Comp. Biochem. Physiol. Vol. 99A, No. 1/2, pp. 207–214, 1991
Printed in Great Britain

0300-9629/91 $3.00 + 0.00
Pergamon Press plc

EFFECT OF PULSATILE OR CONTINUOUS ADMINISTRATION OF PITUITARY-DERIVED CHICKEN GROWTH HORMONE (p-cGH) ON LIPID METABOLISM IN BROILER PULLETS*

R. W. ROSEBROUGH,†‡ J. P. MCMURTRY† and R. VASILATOS-YOUNKEN§

†Nonruminant Animal Nutrition Laboratory, Livestock and Poultry Science Institute, United States Department of Agriculture-Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705, U.S.A. Telephone: 301-344-2866, Fax: 301-344-2623; §Department of Poultry Science, The Pennsylvania State University, University Park, PA 16802, U.S.A. Telephone: 814-465-4642, Fax: 814-465-3103

(*Received* 20 *August* 1990)

Abstract—1. The effects of pulsatile and continuous intravenous administration of exogenous, pituitary-derived chicken growth hormone (p-cGH) on lipid metabolism and endocrine/metabolite levels of broiler-strain pullets were studied.

2. Eight-week-old pullets were administered p-cGH or vehicle over a 10 min period every 90 min for 7 days.

3. Pullets were also administered the same daily amount (123 μg/kg of body weight/day) continuously for 7 days.

4. Feed intake, body weight gain, *in vitro* lipogenesis and hepatic enzyme activities were determined with certain hormones identified with the control of growth.

5. Pulsatile p-cGH administration for 7 days lacked effect on weight gain, feed efficiency, muscle or bone development.

6. Abdominal fat pad size was decreased ($P < 0.05$) by pulsatile but not continuous administration of p-cGH. Pulsatile p-cGH administration also decreased ($P < 0.05$) *in vitro* lipogenesis. Liver malic enzyme and isocitrate dehydrogenase activities were increased ($P < 0.05$) by pulsatile but not continuous administration of p-cGH. In contrast, glutamic oxaloacetic transaminase activity was increased by a continuous infusion of p-cGH.

7. Plasma concentrations of T_4 corticosterone and triglycerides were decreased ($P < 0.05$) by a pulsatile but not a constant infusion of p-cGH.

8. Plasma T_3 and GH were increased ($P < 0.05$) by pulsatile p-cGH compared to both a continuous infusion of p-cGH and the saline controls.

9. This study is the first to prove that in the broiler chicken, the pattern of exogenous p-cGH administration is a factor influencing *in vitro* responses to the hormone.

INTRODUCTION

There are few areas in avian growth and development as controversial as the role of growth hormone (GH) or somatotropin in regulating lean tissue synthesis. For example, work from Gibson and Nalbandov (1966) showed that lean tissue synthesis decreased and fat tissue synthesis increased in the hypophysectomized chicken. More recent work has described the same condition in the turkey (Harvey *et al.*, 1977). The inference of the role of GH on lipid metabolism in the chicken seems to have sprung from interpretations of the actions in mammals, although some reports link GH to a stimulation of lipolysis in chicken adipose tissue (Campbell and Scanes, 1985). Growth hormone depresses *de novo* lipogenesis in pig adipose tissue (Walton and Etherton, 1986; Walton *et al.*, 1986) and stimulates lipolysis in rat adipose tissue (Goodman and Grichting, 1983; Goodman *et al.*, 1986). The role of mammalian lipid metabolism probably involves peripheral resistance to the actions of insulin accompanying GH therapy rather than to an effect of GH *per se*. It can be implied that fat synthesis accompanies normal fat cell metabolism in adipose tissue (Rosebrough and Steele, 1986). Thus peripheral resistance results in an inhibition of glucose transport-phosphorylation and a decrease in certain enzymes required for the support of *de novo* lipogenesis. Of particular importance is a recent report describing the inhibition of the enzymes of the pentose cycle (PC) that produce reduced adenine dinucleotides necessary for *de novo* lipogenesis (Magri *et al.*, 1987).

Recently, GH secretory profiles in broiler pullets have been categorized (Vasilatos-Younken and Zarkower, 1987). Briefly, it can be shown in young, rapidly growing pullets that GH secretory peaks occur at 90 min intervals. In contrast, as the pullets

*Mention of a trade name, proprietary product or specific equipment is not a guarantee or a warranty by the U.S. Department of Agriculture and does not imply an approval to the exclusion of other products.

‡Correspondence should be addressed to, Dr R. W. Rosebrough, NANL-LPSI, Rm 212, Building 200, BARC-EAST, Beltsville, MD 20705, U.S.A.

age, the pulsatile secretory pattern is lost and lean tissue synthesis decreases, followed by a rapid increase in fat synthesis. The secretory pattern noted in the young pullet can be artificially reproduced in the older pullet (Vasilatos-Younken *et al.*, 1988). The result of this regimen is an improvement in feed efficiency and a decrease in apparent fat synthesis.

The objective of the present study was to decide if pituitary derived chicken growth hormone (p-cGH), when administered in an intravenous pulse pattern calculated to mimic the endogenous pattern of GH noted in younger chickens, would significantly alter lipid metabolism in Hubbard × Hubbard broiler pullets during the 8th week of life. This period was chosen because of the propensity of broilers to synthesize fat in preference to protein during this part of the growth phase.

MATERIALS AND METHODS

Animals and in vivo *infusions*

Chickens used in this study were Hubbard × Hubbard pullets hatched and reared at the Pennsylvania State University Poultry Research Facility. Chickens were fed a commercial broiler starter (21% crude protein; 3135 kcal ME/kg calculated analysis) *ad libitum* throughout the growth cycle, except during the 24 hr immediately before surgery as noted. Chickens were maintained under a 16:8 hr (light:dark) for the entire experiment. At 53 days of age, birds were prepared with intravenous (right jugular) catheters under sodium pentabarbital anesthesia according to previously described methods (Cravener and Vasilatos-Younken, 1989).

Pituitary-derived chicken growth hormone was purified as previously described (Cravener *et al.*, 1989). The hormone was dissolved in a sterile, buffered saline solution (vehicle) containing 0.025 M $NaHCO_3$ plus 0.025 M Na_2CO_3, pH 9.4 (Peel *et al.*, 1981), which also served as the control solution for infusion. Beginning at 8 weeks of age, four birds each were intravenously infused with either p-cGH or vehicle in either a pulsatile (70 μl/pulse; 10 min/pulse duration; 80 min/interpulse interval; 16 pulses/24 hr) or continuous (50 μl/hr) pattern, 24 hr per day for 7 consecutive days. This entire experiment was replicated twice for a total of 32 birds (eight birds per hormone × pattern treatment combination). The concentration of growth hormone solutions was calculated such that a dosage of 120 μg/kg body weight/day was administered to all p-cGH infused birds, regardless of pattern.

Body weights were determined immediately before and at the termination of infusions for calculation of net body weight gain. Feed intake was measured daily. Blood samples were removed from each bird by (brachial) venipuncture immediately before initiation of infusions and after the 7-day infusion period, during both peak (last one min of a 10-min pulse) and baseline (last one min of an 80 min inter-peak interval) periods. Sodium citrate was used as an anticoagulant (5 μl of a 50% solution per 1.0 ml whole blood) and plasma obtained by centrifugation. Sodium chloride was added to plasma (20 mg/ml plasma) to inhibit endogenous lipoprotein lipase activity. Following final blood sampling, each bird was killed by cervical dislocation, and GH-responsive organs (liver, heart, kidney, pancreas, spleen, abdominal fat pad, right tibiotarsas and tibiotarsal ephiphyseal growth plate) rapidly removed, weighed, and subsamples frozen in liquid nitrogen. Organ and plasma samples were stored at −70°C until analysed.

In vitro *lipogenesis*

Livers were excised and washed initially with PBS to remove blood and debris and then placed in fresh, chilled PBS. A portion of liver was also frozen in liquid N_2 and stored at −70°C. Pieces of fresh livers were sliced with a MacIlwain tissue chopper (35–75 mg) at a setting corresponding to a thickness of 0.3 mm. The explants were placed in 75 mm Petri dishes containing chilled PBS and randomly allocated to *in vitro* treatments. Quadruplicate explants were incubated at 37° for 2 hr in Hanks' balanced salts (Hanks and Wallace, 1949) supplemented with 10 mM HEPES and 10 mM [2-^{14}C]sodium acetate (Rosebrough and Steele, 1987). All incubations were conducted in 3 ml volumes at 37°C under a 95% O_2–5% CO_2 atmosphere (obtained by gassing vessels for 30 sec). After the 2 hr period, incubations were stopped and the [^{14}C]CO_2 trapped in the buffer was released by addition of 0.25 ml of 4 N H_2SO_4, followed by an additional 1 hr incubation period to allow for [^{14}C]CO_2 absorption onto filter papers. Filter papers were removed, placed in 20 ml of scintillation cocktail, allowed a 24 hr equilibration period, and counted. Slices were removed from incubation flasks and extracted for 24 hr in 15 ml of a 2:1 mixture of chloroform:methanol in 20 ml glass scintillation vials. Slices were then discarded and 2 ml of 117 mM KCl added to the chloroform:methanol extracts (Folch *et al.*, 1957). After vigorous shaking, phase separation was allowed and the upper phases discarded. The lower phase was washed with 5 ml of a 3:2 mixture of methanol:117 mM KCl, shaken and again allowed to separate. The upper phase was discarded and the lower phase evaporated to dryness, dispersed in 10 ml scintillation cocktail and counted.

Enzyme assays

Liver tissue was homogenized (1:10 wt/vol) in 100 mM HEPES (pH 7.5)–3.3 mM mercaptoethanol and centrifuged (Beckman J2-21, Beckman Instruments, Inc., Palo Alto, CA 94304) at 50,000 *g* for 60 min. The supernatants were kept at 0°C until analysed for ME, ICD, GOT and TAT. ME activity was determined by a modification of the method of Hsu and Lardy (1969). The reaction contained 50 mM HEPES (pH 7.5), 1 mM NADP, 5 mM $MnCl_2$ and the substrate, 2.2 mM L-malate (disodium salt). A 50 μl aliquot of the 50,000 *g* supernatant was preincubated for 15 min in the presence of the first three ingredients. The reaction was initiated by adding L-malate and following the rate of reduction of NADP at 340 nm at 25°C. The reaction was found to proceed linearly for at least 60 min providing that the reaction contained no more than 100 μg of supernatant protein.

ICD activity was determined by a modification of the method of Cleland *et al.* (1969). The reaction contained 50 mM HEPES (pH 7.5), 1 mM NADP, 5 mM $MnCl_2$ and the substrate, 4.4 mM DL-isocitrate. A 25 μl aliquot of the 50,000 *g* supernatant was preincubated for 15 min in the presence of the first three ingredients. The reaction was initiated by adding DL-isocitrate and following the rate of reduction of NADP at 340 nm at 25°C. The reaction was found to proceed linearly for at least 60 min providing that the reaction contained no more than 50 μg of supernatant protein.

GOT activity was determined by a modification of the method of Martin and Herbein (1976). The reaction contained 50 mM HEPES, 200 mM L-aspartate, 0.2 mM NADH, 1000 units per liter malic dehydrogenase and the substrate, 15 mM α-ketoglutarate. A 25 μl aliquot of the 50,000 *g* supernatant was preincubated for 15 min in the presence of the first four ingredients. The reaction was initiated by adding α-ketoglutarate and following the rate of oxidation of NADH at 340 nm at 25°C. The reaction was found to proceed linearly for at least 30 min providing that reaction contained no more than 50 μg of supernatant protein.

TAT activity was determined by the method of Granner and Tomkins (1970). The reaction contained 0.125 M K_2HPO_4 (pH 7.4), 6 mM L-tyrosine, 10 mM pyridoxal phosphate (PLP) and 50 mM α-ketoglutarate. A 200 μl aliquot of the supernatant was preincubated at 37°C in the presence of the first three ingredients. The reaction was then initiated by adding α-ketoglutarate and allowing the reaction to proceed for 15–30 min. The reaction was stopped with 10 M NaOH and the subsequent product, p-hydroxybenzaldehyde, was monitored at 331 nm. Enzyme activities are expressed as μmol of product formed/min under the assay conditions (Rosebrough and Steele, 1985).

Hormone and metabolite assays

Both T_3 and T_4 concentrations were estimated with commercial, solid-phase kits (Immuchem Corp, Carson, CA). These assays were validated for avian samples (Rosebrough *et al.*, 1988) by dispersing standards in charcoal-stripped chicken serums and by noting recovery of added T_3 and T_4 (98%). All hormone assays were conducted as single batches to minimize intra-assay variation. The inter-assay coefficients of variation averaged 3.7 and 1.1% for T_3 and T_4 respectively.

Plasma GH concentration was estimated with a homologous chicken GH radioimmunoassay (RIA) using recombinant (r-) chicken GH (AMGEN, Thousand Oaks, CA; Lot 001) as a standard and for iodination, and a rabbit anti-r-chicken GH serum as primary antibody (Vasilatos-Younken, 1986). Plasma IGF-1 was estimated with a heterologous radioimmunoassay as previously described (Dawe *et al.*, 1988). ^{125}I-labeled IGF-I was purchased from Amersham Corp and purified human sequence IGF-I for standard was supplied by Bachem, Torrance, CA. Primary antisera (rabbit anti-human IGF-I) was kindly provided by Dr Geoff Francis, C.S.I.R.O., Adelaide, S.A. Plasma glucose, triglycerides, nonesterified free fatty acids, and corticosterone were determined with commercially available kits (Sigma Chemical Bulletin Nos. 16UV and 335-B, Sigma Chemical Co., St Louis, MO; NEFA-C, Wako Pure Chemical Industries, Ltd, Osaka, Japan; Cambridge Medical Diagnostics Inc., Billerica, MA, respectively).

Liver malate was determined by extracting liver according to Rosebrough *et al.* (1981) and measuring malate by the method of Gutman and Wahlefeld (1974). One-gram of liver was homogenized in 10 ml of 1 M $HClO_4$ and centrifuged at 50,000 g for 15 min to provide a protein-free supernatant. Three-milliliter aliquots of the supernatant were neutralized with 0.6 ml of 5 M K_2CO_3. Aliquots of the neutralized extract were incubated at 25°C for 30 min in the presence of 0.5 M glycine-0.4 M hydrazine (pH 9.0) containing 1 mM NAD and 1000 units/l of malic dehydrogenase. The reduction of NAD was followed at 340 nm and was assumed to be proportional to that amount of malate present in the reaction mixture.

Statistical design and analyses

Pre-infusion body weight (PreBW) was used as a covariate in analyses of variance of data according to the model: Y = PreBW (covariate), experiment replicate (1 or 2), hormone (p-cGH or control), pattern (pulsatile or continuous), hormone × pattern interaction, replicate × hormone and replicate × pattern. All main effects and interactions were tested against residual error. Where any replicate × treatment interaction was not significant, that interaction was dropped from the model and the data reanalysed using the reduced model. Where any replicate × treatment interaction was significant, that interaction was used as the error term to test for a significant main effect (e.g. a significant replicate × hormone interaction was used as the error term for testing the main effect of hormone). Percentage data (e.g. organs as a percentage of body weight) were transformed to arcsin square root before statistical analysis. All data are expressed as least squares means (LSM). The general linear models procedure (GLM) of SAS (1982) was used for derivation of transformed data, least squares means, and mean square components.

RESULTS

An initial series of analyses was conducted to decide if replication × treatment interactions were significant. Interactions were not significant for any of the parameters measured; therefore, all data were pooled across replications. Also, analyses were conducted to decide significance between means for the two delivery methods for saline. In nearly all cases, the method of delivering saline was not significant; therefore, saline means were pooled to develop a common control to assess the effects of the two methods for delivering p-cGH. Data for pulsatile treatments were considered only if pulse values for GH were 2 standard deviations above control values. Average, initial body weights before initiation of infusions were nearly identical for p-cGH and controls treatments. No measurement of live growth performance (weight gain, feed intake or efficiency) was significantly different among treatments after the 7-day treatment period (Table 1). Both p-cGH administration methods increased ($P < 0.05$) kidney size on a mass and relative size basis (Table 2). Livers of chickens infused continuously with p-cGH were larger ($P < 0.05$) than controls both on an absolute and relative mass basis ($P < 0.05$). Neither pulsatile nor continuous administration of p-cGH changed muscle weights (pectoralis major and gastrocnemius), organ sizes (pancreas, heart and pituitary) or bone growth (epiphyseal growth plate width and length and weight of the tibiotarsus).

The abdominal fat pad, which is a major source of carcass fat in poultry and an excellent predictor of general fatness, was decreased 32% in chickens administered pulsatile-p-cGH in comparison to controls (Table 3). In contrast, the fat pad was reduced numerically, but not statistically in chickens given p-cGH continuously. Pulsatile, but not continuous administration of p-cGH markedly decreased

Table 1. Effect of 7-d intravenous pulsatile or continuous infusion of pituitary-derived chicken growth hormone (p-cGH) or buffered saline (control) on growth of broiler pullets*

Parameter	p-cGH-pulsatile	p-cGH-continuous	Saline
Initial body weight (g)	2429.0 ± 76.2†	2429.0 ± 76.7†	2424.0 ± 46.9†
7-d weight gain (g)	268.3 ± 72.3†	306.8 ± 80.9†	369.4 ± 31.7†
7-d feed intake (g)	1167.0 ± 80.0†	1280.0 ± 89.0†	1246.0 ± 41.0†
Feed efficiency (gain/g feed consumed)	0.21 ± 0.05†	0.23 ± 0.06†	0.29 ± 0.03†

*Eight-week-old female broiler chickens were intravenously infused with either p-cGH or vehicle in either a pulsatile (70 μl/pulse; 10 min/pulse duration; 80 min/interpulse interval; 16 pulses/24 hr) or continuous (50 μl/hr) pattern, 24 hr per day for 7 consecutive days. Least squares means (LSM) ± standard error of LSM. $n = 9$ continuous/control; 4 continuous/p-cGH; 10 pulsatile/control; 5 pulsatile/p-cGH.

†Means within a row without common superscripts differ ($P < 0.05$) as determined by a t-test.

Table 2. Effect of 7-d intravenous pulsatile or continuous infusion of pituitary-derived chicken growth hormone (p-cGH) or buffered saline (control) on skeletal and organ parameters of broiler pullets*

Parameter	p-cGH-pulsatile	p-cGH-continuous	Saline
Pectoralis major			
Weight (g)	377.3 ± 28.5†	364.5 ± 31.8†	411.2 ± 9.9†
% final live weight	13.8 ± 0.5†	13.2 ± 0.6†	14.6 ± 0.3†
Right gastrocnemius			
Weight (g)	10.7 ± 0.7†	10.5 ± 0.8†	11.5 ± 0.3†
% final live weight × 10	3.9 ± 0.2†	3.8 ± 0.2†	4.1 ± 0.1†
Heart			
Weight (g)	11.7 ± 0.9†	11.1 ± 0.9†	10.4 ± 0.3†
% final live weight × 10	4.3 ± 0.3†	4.1 ± 0.4†	3.8 ± 0.1†
Kidney			
Weight (g)	23.9 ± 1.1‡	25.5 ± 1.2‡	19.8 ± 0.4†
% final live weight × 10	8.7 ± 0.3‡	9.4 ± 0.4‡	7.1 ± 0.2†
Liver			
Weight (g)	68.7 ± 4.1†‡	76.9 ± 4.6‡	60.7 ± 2.1†
% final live weight	2.6 ± 0.1†‡	2.8 ± 0.2‡	2.1 ± 0.1†
Pancreas (g)	5.1 ± 0.5†	5.9 ± 0.5†	5.4 ± 0.2†
Pituitary (mg)	7.1 ± 0.5†	7.9 ± 0.6†	7.5 ± 0.4†
Epiphyseal growth plate			
Width (mm)	1.1 ± 0.1†	0.9 ± 0.1†	1.0 ± 0.1†
Right tibiotarsus			
Length (mm)	134.1 ± 1.9†	135.5 ± 2.1†	137.0 ± 0.9†
Weight (g)	23.7 ± 1.3†	25.9 ± 1.4†	22.9 ± 0.4†

*Eight-week-old female broiler chickens were intravenously infused with either p-cGH or vehicle in either a pulsatile (70 μl/pulse; 10 min/pulse duration; 80 min/interpulse interval; 16 pulses/24 hr) or continuous (50 μl/hr) pattern, 24 hr per day for 7 consecutive days. Least squares means (LSM) ± standard error of LSM. $n = 9$ continuous/control; 4 continuous/p-cGH; 10 pulsatile/control; 5 pulsatile/p-cGH.

†‡Means within a row without common superscripts differ ($P < 0.05$) as determined by a t-test.

($P < 0.05$) *in vitro* lipogenesis compared to control. The lipogenic rates in both controls and continuously infused chickens were nearly identical. These trends were noted when data were expressed per unit of liver weight and per 100 g body weight. Carbon dioxide production was decreased ($P < 0.05$) by pulsatile, but not continuous p-cGH administration.

Liver malate, which is a major source of reducing potential for *de novo* lipogenesis, was decreased ($P < 0.05$) whereas ICD was increased ($P < 0.05$) by pulsatile, but not continuous administration of p-cGH, when compared to saline controls (Table 4). Malic enzyme activity was increased ($P < 0.05$) by pulsatile administration of p-cGH compared to a continuous administration of p-cGH and to saline controls. Tyrosine amino transferase activity was increased ($P < 0.05$) by a continuous administration of p-cGH compared to pulsatile administration of p-cGH or to the saline controls.

Except for plasma NEFA, all pre-infusion metabolite concentrations did not differ between treatment groups (data not shown). Pulsatile p-cGH administration had significant effects on plasma hormone concentrations (Table 5). On the final day of infusions, CGH concentrations were higher ($P < 0.05$) for chickens infused with p-cGH than for controls. Plasma GH was much higher after the pulse period than at basal levels, confirming the adequacy of our protocol for inducing pulsatile changes in cGH. Plasma immunoreactive IGF-I was slightly lower ($P < 0.05$) in controls than in chickens continuously infused with p-cGH and not different from both the basal and pulse period of the chickens given p-cGH in a pulsatile manner. Plasma concentrations of T_4 and triglycerides were decreased ($P < 0.05$) by a pulsatile but not a constant infusion of p-cGH whereas corticosterone was markedly increased by continuous p-cGH ($P < 0.05$). Plasma T_3 and GH

Table 3. Effect of 7-d intravenous pulsatile or continuous infusion of pituitary-derived chicken growth hormone (p-cGH) or buffered saline (control) on abdominal fat pad development and *in vitro* lipogenesis of female broiler pullets*†

Parameter	p-cGH-pulsatile	p-cGH-continuous	Saline
Abdominal fat pad			
Weight (g)	45.1 ± 4.7‡	60.1 ± 9.2‡§	66.8 ± 4.6§
% final body weight	1.67 ± 0.15‡	2.24 ± 0.43§	2.37 ± 0.15§
In vitro metabolism			
Lipogenesis			
nmol/100 mg liver	140.7 ± 39.0‡	728.9 ± 174.1§	752.0 ± 84.2§
μmol/100 g body weight	3.4 ± 0.9‡	19.5 ± 4.6§	16.2 ± 1.9§
CO_2 production			
nmol/100 mg liver	234.2 ± 57.3‡	491.6 ± 21.5§	409.9 ± 43.9§
μmol/100 g body weight	5.8 ± 1.3‡	13.9 ± 1.4§	8.7 ± 0.9§

*Eight-week-old female broiler chickens were intravenously infused with either p-cGH or vehicle in either a pulsatile (70 μl/pulse; 10 min/pulse duration; 80 min/interpulse interval; 16 pulses/24 hr) or continuous (50 μl/hr) pattern, 24 hr per day for 7 consecutive days. Least squares means (LSM) ± standard error of LSM. $n = 9$ continuous/control; 4 continuous/p-cGH; 10 pulsatile/control; 5 pulsatile/p-cGH.

†*In vitro* lipogenesis and CO_2 were determined by culturing liver explants for 2 hr in the presence of 10 mM [2-^{14}C]sodium acetate and by noting incorporation of acetate into hepatic lipids or CO_2. Both processes are noted as nmol of acetate incorporated/100 mg of liver or μmol/100 g body weight.

‡§Means within a row without common superscripts differ ($P < 0.05$) as determined by a t-test.

Table 4. Effect of 7-d intravenous pulsatile or continuous infusion of pituitary-derived chicken growth hormone (p-cGH) or buffered saline (control) on hepatic malate levels and enzyme activities of broiler pullets*†

Parameter	p-cGH-pulsatile	p-cGH-continuous	Saline
Malate			
nmol/g liver	2.9 ± 0.4†	5.8 ± 0.4§	5.0 ± 0.2§
GOT			
units/g liver	70.1 ± 9.3‡	97.8 ± 10.7§	62.4 ± 3.7‡
units/100 g body weight	174.4 ± 18.3‡	242.8 ± 8.1§	134.8 ± 8.5‡
ICD			
units/g liver	42.7 ± 8.4§	37.9 ± 7.9‡§	30.4 ± 3.4‡
units/100 g body weight	108.3 ± 16.4§	102.2 ± 18.7‡	66.2 ± 7.7‡
ME			
units/g liver	11.4 ± 2.4§	4.8 ± 1.0‡	4.9 ± 0.5‡
units/100 g body weight	28.8 ± 5.1§	13.0 ± 2.1‡	10.9 ± 1.3‡
TAT			
units/g liver	3.5 ± 0.1‡	4.7 ± 0.4§	3.6 ± 0.2‡
units/100 g body weight	9.1 ± 0.5‡	13.3 ± 1.5§	7.8 ± 0.5‡

*Eight-week-old female broiler chickens were intravenously infused with either p-cGH or vehicle in either a pulsatile (70 μl/pulse; 10 min/pulse duration; 80 min/interpulse interval; 16 pulses/24 hr) or continuous (50 μl/hr) pattern, 24 hr per day for 7 consecutive days. Least squares means (LSM) ± standard error of LSM. $n = 9$ continuous/control; 4 continuous/p-cGH; 10 pulsatile/control; 5 pulsatile/p-cGH.

†GOT = glutamic oxaloacetic aminotransferase (EC 2.6.1.1); ICD (NADP) = isocitrate dehydrogenase (NADP) (EC 1.1.1.42); ME = malic enzyme (EC 1.1.1.40); TAT = tyrosine amino transferase (EC 2.6.1.5). Activities are expressed as μmol of reduced or oxidized NADP(H) or product formed per minute under standard assay conditions.

‡§Means within a row without common superscripts differ ($P < 0.05$) as determined by a t-test.

were increased ($P < 0.05$) by pulsatile p-cGH compared to both a continuous infusion of p-cGH and the saline controls. Plasma NEFA concentrations were highly variable among individual animals before the experimental treatment administration (range = 103.9 to 800.6 μEq/l), therefore, post-infusion data were expressed as percentage change from pre-infusion concentrations for each individual animal, and percentage change data then averaged across animals, within treatment groups. Peak values for the change due to pulsatile infused p-cGH were greater than the change noted in the continuously infused birds. Continuous infusion of p-cGH depressed NEFA that contrasted to the increase noted in the pulsatile group. Plasma glucose was not significantly different among treatments.

DISCUSSION

The length of treatment used in the present experiment was chosen so that changes in parameters of lipid metabolism would be expressed before the changes in growth (e.g. skeletal) that have been previously noted (Vasilatos-Younken *et al.*, 1988). It is possible that long-term treatment protocols may produce changes in metabolic parameters that are secondary to changes in gross morphology. In addition, it has also been noted that voluntary feed intake is depressed in chickens treated with p-cGH for periods longer than 1 week (Vasilatos-Younken *et al.*, 1988). The results of the present study confirm that the pattern of p-cGH delivery determines whether the hormone will affect lipid metabolism like growth and body composition. It has been previously shown that a continuous delivery of p-cGH to the chicken did not enhance skeletal development or suppress adipose tissue synthesis (Vasilatos-Younken *et al.*, 1988). In this context, it is interesting to examine several recent studies that suggest that p-cGH has small or no effect on growth of chickens. A recent study showed that daily subcutaneous injections of p-cGH for 11 days did not increase heat production of chickens (Cogburn *et al.*, 1989a). Also, this group found, surprisingly, that

Table 5. Effect of 7-d intravenous pulsatile or continuous infusion of pituitary-derived chicken growth hormone (p-cGH) or buffered saline (control) on plasma hormones and metabolites of broiler pullets*†

Parameter	p-cGH-pulsatile Peak	p-cGH-pulsatile Baseline	p-cGH-continuous	Saline
GH (ng/ml)	46.4 ± 4.0‡	1.6 ± 0.6§	14.7 ± 0.6¶	1.7 ± 1.1¶
IGF-1 (ng/ml)	18.2 ± 1.4§¶	18.4 ± 1.4§¶	20.2 ± 1.4¶	16.2 ± 1.0§
T_3	3.6 ± 0.3‡	3.2 ± 0.3¶‡	2.6 ± 0.3¶	1.7 ± 0.2§
T_4	4.2 ± 1.3§	4.1 ± 0.9§	7.4 ± 1.3¶	9.5 ± 0.7¶
Corticosterone (ng/ml)	3.7 ± 2.4§	6.5 ± 3.2§	21.3 ± 3.8¶	6.4 ± 2.4§
NEFA (% change from preinfusing concentrations)	22.5 ± 14.5§	39.3 ± 24.4§	−28.2 ± 26.8§	9.7 ± 15.4§
Glucose (mg/100 ml)	230.0 ± 13.3§	217.0 ± 12.7§	238.0 ± 14.6§	226.0 ± 10.1§
Triglycerides (mg/100 ml)	31.7 ± 4.3§	48.7 ± 5.8¶	51.2 ± 4.6¶	54.8 ± 3.2¶

*Eight-week-old female broiler chickens were intravenously infused with either p-cGH or vehicle in either a pulsatile (70 μl/pulse; 10 min/pulse duration; 80 min/interpulse interval; 16 pulses/24 hr) or continuous (50 μl/hr) pattern, 24 hr per day for 7 consecutive days. Least squares means (LSM) ± standard error of LSM. $n = 9$ continuous/control; 4 continuous/p-cGH; 10 pulsatile/control; 5 pulsatile/p-cGH.

†GH = growth hormone; IGF-I = insuline-like growth factor I; T_3 = triiodothyronine; T_4 = thyroxine; NEFA = non-esterified fatty acids. Plasma NEFA concentrations before initiation of infusions differed significantly between treatment groups, therefore, post-infusion concentrations are expressed relative to pre-infusion concentrations within treatments.

‡§¶Means within an infusion pattern without common superscripts differ between GH and control treatments ($P < 0.05$) as determined by a t-test.

daily subcutaneous injections of 100–200 μg of p-cGH significantly increased body fat of chickens. Scanes (1987) took a different approach in attempting to improve growth of hypophysectomized chickens with chicken p-cGH, but did not succeed with their therapy program. Leung *et al.* (1986) found that intravenously administered p-cGH caused a transient improvement in chicken growth. In contrast, p-cGH did not influence the growth of layer strain chickens when administered subcutaneously (Scanes *et al.*, 1986).

In attempting to reconcile the discordant responses to exogenously administered cGH in chickens observed among studies, and perhaps even build a model for GH action from the sum all such work, some necessary distinctions must be recognized. Given the pulsatility of endogenous circulating GH in poultry, chronic exposure to GH might be defined as an elevation in circulating GH concentration (above baseline) of greater than one pulse interval (approximately 90 min). Administration regimens involving subcutaneous injection of the hormone clearly elevate plasma GH well beyond such durations (Cogburn *et al.*, 1989b; Burke *et al.*, 1987). Delineation of studies into categories of chronic (subcutaneous injection; continuous intravenous infusion) vs acute (intravenous injection or pulsatile intravenous infusion) exposure begin to suggest a scheme by which the apparent actions of GH, particularly in terms of lipid metabolism and carcass fat deposition, might be reconciled. Such a scheme involves assessment of alterations in metabolic regulatory hormones in response to chronic vs acute exposure.

Chronic GH significantly circulating corticosterone (present work) and insulin concentrations (Vasilatos-Younken *et al.*, 1988; Burke *et al.*, 1987); or numerically but not significantly elevated insulin and decreased insulin/glucagon ratios (Cogburn *et al.*, 1989), whereas acute GH did not (Burke *et al.*, 1987; present work). Corticosterone administration markedly increases carcass fat deposition (Gross *et al.*, 1980; Davison *et al.*, 1983) and *in vitro* lipogenesis (Kafri *et al.*, 1988) in pituitary-intact chickens. Insulin is necessary for maintenance of high rates of lipogenesis by hepatocytes from fed chickens in culture (Tarlow *et al.*, 1977), and prolonged exposure to glucocorticoids potentiates insulin-dependent hepatic lipogenesis and increases plasma insulin in the rat (Amatruda *et al.*, 1983). Responses in fat deposition and lipid metabolism with acute vs chronic GH are equally divergent. Acute GH reduced abdominal fat pad size, carcass fat content and hepatic lipogenesis (Vasilatos-Younken, 1988; present work), whereas chronic GH generally lacked significant effect (Cravener *et al.*, 1989; Cogburn *et al.*, 1989a) or increased carcass fat (Cogburn *et al.*, 1989b; Burke *et al.*, 1987). Consistent with the divergent responses in (1) fat deposition/lipid metabolism and; (2) profiles of corticosterone and insulin with chronic vs acute GH, is the observation that GH alone lacked effect, but GH plus corticosterone greatly increased abdominal fat pad weight in hypophysectomized chickens (King and Scanes, 1986).

Another facet of this scheme may involve relative responses in thyroid hormones to acute vs chronic GH administration. Acute GH increases circulating T_3 concentrations (Vasilatos-Younken *et al.*, 1988), whereas chronic GH depresses T_3 (Marsh *et al.*, 1984); lacks no effect (Vasilatos-Younken *et al.*, 1988); or increases concentrations but to a much lesser degree than acute exposure (present data). Chronic GH plus supplemental T_3 markedly reduced carcass fat (beyond T_3 alone) (Cogburn *et al.*, 1989b) and acute GH plus supplemental T_3 eliminated *in vitro* hepatic lipogenesis (Rosebrough and Vasilatos-Younken, unpublished data).

While the mechanisms of action of p-cGH are not fully understood, it is known that this hormone directly influences metabolism in some cells and operates through insulin-like growth factors (IGF I and II). Although there is much information available concerning biochemical aspects of these polypeptide hormones, there is an absence of information regarding their functions in rapidly growing chickens. The direct influence of p-cGH on lipid metabolism in adipose tissue *in vitro* has been extensively studied in the rat (Goodman, 1983, 1984; Grichting and Goodman, 1986) and in the pig (Chung *et al.*, 1985; Chung and Goodman, 1986; Etherton *et al.*, 1987). These studies have shown that p-cGH inhibits lipogenesis and stimulates lipolysis; observations that may provide a partial explanation for the reduction in body fat in GH-treated mammals. Even in these animals, it should be emphasized that the observed reductions in body fat cannot be totally explained by GH therapy and suggest a need for investigation of other regulatory mechanisms. For example, it has been sugested that normal circulating levels of GH are sufficient to attain maximal GH effects in adipose tissue (Goodman and Grichting, 1983; Goodman *et al.*, 1986). It appears that *in vitro* effects also may require glucocorticoids as permissive agonists. The latter observation can be supported by examining long-term culture systems that use swine adipose tissue to determine the role of GH in the regulation of *de novo* lipogenesis (Walton and Etherton, 1986; Walton *et al.*, 1986).

The reduction in plasma T_4 in the current study seems to support the hypothesis of an increase in peripheral conversion of thyroxine to triiodothyronine in chickens administered p-cGH in a pulsatile manner. Previous research showed that liver 5′-monodeiodinase activity was increased by p-cGH (Kuhn *et al.*, 1987). Certainly, the levels of T_3 noted in chickens administered pulsatile p-cGH suggest an enhanced conversion of T_4 to T_3. It is uncertain now if liver 5′-monodeiodinase catalyzes a rate-limiting reaction or if enzyme activity reflects an increase in the demand for thyroid hormones in other tissues or to a change in T_3 turnover. Scanes *et al.* (1982) and May and Marks (1983) measured the thyroid hormones in both dwarf and normal chickens and found elevated T_4 levels in dwarfs and hypothesized changes in peripheral conversion modulated by GH. Both groups indicated that the conversion of T_4 to T_3 was deficient in dwarf strains of chickens, which are animals with known perturbations in GH metabolism.

The observation of increased ME activity in chickens administered p-cGH in a pulsatile fashion may suggest some effects attributed to p-cGH are results of increases in thyroid metabolism. Initial work by Oppenheimer *et al.* (1978) showed a positive correlation between T_3 level and hormone action at the cellular level. According to their hypothesis, a decrease in either tissue binding or circulating levels of T_3 would decrease ME activity. Stewart *et al.* (1984) reported depressed T_3 levels in dwarf chickens and speculated that this depression was in some way related to ME activity. The apparent dichotomy noted in ME activity in the present study presents a challenge in determining roles of enzymes thought to be synonymous with lipogenesis. In the present study, pulsatile administration of p-cGH depressed both lipogenesis and a representative site of fat deposition, the abdominal fat pad. In the process of this decrease, ME activity was increased. We are unaware of any studies concerning dietary factors and lipogenesis in broiler chickens that do not show a positive relationship between ME activity and *de novo* lipogenesis. Also, in our hands, most dietary regimens that decrease lipogenesis such as high-protein diets and fasting increase the activities of TAT, GOT and ICD that suggest an increase in protein catabolism. Such was not so in the present study. On the other hand, p-cGH did decrease liver malate that may be a source of NADPH for *de novo* fatty acid synthesis.

In summary, our study shows that a pulsatile, intravenous pattern of hormone delivery chosen to mimic the endogenous pattern of GH in young, rapidly growing chickens will decrease *de novo* lipogenesis. Also, this decrease in lipogenesis is accompanied by a decrease in abdominal fat pad weight. These changes in lipid metabolism appear to precede earlier noted changes in skeletal morphology.

A pattern emerges in which chronic GH administration is associated with enhanced corticosterone and insulin status and depressed or modestly increased T_3. These patterns are with either no significant effect on net carcass fat deposition and hepatic lipogenesis, or increased carcass fat. In contrast, acute GH is associated with no increase in corticosterone or insulin, and marked elevations in circulating T_3 concentrations, with reduced carcass fat deposition and *in vitro* hepatic lipogenesis. With the additional observation that GH stimulates adipose tissue lipolysis (Campbell and Scanes, 1985), these data collectively suggest a biological effect for GH in the chicken of reducing net adipose tissue deposition via reduced hepatic lipogenesis and (possibly) enhanced adipose tissue lipolysis, but, chronic exposure to the hormone may result in alterations in other metabolic regulatory hormones known to influence adipogenesis.

REFERENCES

Amatruda J. M., Danahy S. A. and Chang C. L. (1983) The effects of glucocorticoids on insulin-stimulated lipogenesis in primary cultures of rat hepatocytes. *Biochem. J.* **212,** 135–141.

Burke W. H., Moore J. A., Goez J. R. and Builder S. E. (1987) The properties of recombinant chicken growth hormone and its effects on growth, body composition, feed efficiency, and other factors in broiler chickens. *Endocrinology* **120,** 651–658.

Campbell R. M. and Scanes C. G. (1985) Lipolytic activity of purified pituitary and bacterially derived growth hormone on chicken adipose tissue *in vitro*. *Proc. Soc. Exp. Biol. Med.* **180,** 513–517.

Chung C. S., Etherton, T. D. and Wiggins J. P. (1985) Stimulation of swine growth by porcine growth hormone. *J. Anim. Sci.* **60,** 118–130.

Chung C. S. and Etherton T. D. (1986) Characterization of porcine growth hormone (pGH) binding to porcine liver microsomes. Chronic administration of pGH induced pGH binding. *Endocrinol.* **119,** 780–786.

Cleland W. W., Thompson V. M. and Barden R. E. (1969) Isocitrate dehydrogenase (TPN specific) from pig heart. In *Methods in Enzymology* (Edited by Lowenstein, J. M.), Vol. 13, pp. 30–33. Academic Press, New York.

Cogburn L. A., Liou S. S., Rand A. and McMurtry J. P. (1989a) Growth, metabolic and endocrine responses of broiler cockerals given a daily subcutaneous injection of natural or biosynthetic chicken growth hormone. *J. Nutr.* **119,** 1213–1222.

Cogburn L. A., Liou S. S. and McGuiness M. C. (1989b) A novel hormonal treatment that dramatically reduces fat content of broiler chickens. *Poultry Sci.* **68** (Suppl. 1), 33.

Cravener T. L. and Vasilatos-Younken R. (1989) A method for catheterization, harnessing and chronic infusion of undisturbed chickens. *Lab. An.* **23,** 270–274.

Cravener T. L., Vasilatos-Younken R. and Wellenreiter R. H. (1989) Effect of subcutaneous infusion of pituitary-derived chicken growth hormone on growth performance of broiler pullets. *Poultry Sci.* **68,** 1133–1140.

Davison T. F., Rea J. and Rowell J. G. (1983) Effects of dietary corticosterone on the growth and metabolism of immature *Gallus domesticus*. *Gen. Comp. Endocrinol.* **50,** 463–468.

Dawe S. R., Francis G. L., McNamara P. J., Wallace J. C. and Ballard P. J. (1988). Purification, partial sequences and properties of chicken insulin-like growth factors. *J. Endocrinol.* **117,** 173–181.

Etherton T. D., Wiggins P. J., Evock C.M., Chung C. S., Rebhun J. F., Walton P. E. and Steele N. C. (1987) Stimulation of pig growth performance by porcine growth hormone. Determination of the dose–response relationship. *J. Anim. Sci.* **64,** 433–443.

Folch J., Lees M. and Sloane-Stanley G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509.

Gibson W. R. and Nalbondov A. V. (1966) Lipolysis and lipogenesis in liver and adipose tissue of hypophysectomized cockerals. *Am. J. Physiol.* **211,** 1352–1356.

Goodman H. M. (1968) Effect of growth hormone on the lipolytic response of adipose tissue to theophylline. *Endocrinol.* **82,** 1027–1034.

Goodman H. M. (1984) Biological activity of bacterially derived human growth hormone in adipose tissue of hypophysectomized rats. *Endocrinology* **114,** 131–135.

Goodman H. M., Gorin E. and Honeyman T. W. (1986) Biochemical basis for the lipolytic activity of growth hormone. In *Perspectives in Growth Hormone Research* (Edited by Sherman B. and Underwood L.). Marcel Dekker, New York.

Goodman H. M. and Grichting G. (1983) Growth hormone and lipolysis. A re-evaluation. *Endocrinol.* **113,** 1111-1120.

Granner D. K. and Tomkins G. M. (1970) Tyrosine amino transferase. In *Methods in Enzymology* (Edited by Lowenstein, J. M.), Vol. 17, pp. 6343–637. Academic Press, New York.

Grichting G. and Goodman H. M. (1986) Growth hormone maintains its own receptors in rat adipose tissue. *Endocrinology* **119,** 847–854.

Gross W. B., Siegel P. B. and Duboise R. T. (1980) Some effects of feeding corticosterone to chickens. *Poultry Sci.* **59,** 516–522.

Gutman I. and Wahlefeld A. U. (1974) L-(-)-malate determination with malate dehydrogenase and NAD. In *Methods of Enzymatic Analysis* (Edited by Bergmeyer H. U.), Vol. 3, pp. 1585–1589, Academic Press, New York.

Hammond J. A. (1952) Physiological limits to intensive production in animals. *Brit. Agric. Bull.* **4**, 222–225.

Hanks J. H. and Wallace R. E. (1949) Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proc. Soc. Exp. Biol. Med.* **71**, 196–200.

Harvey S., Scanes C. G. and Howe T. (1977) Growth hormone effects on *in vitro* metabolism of avian adipose and liver tissue. *Gen. Comp. Endocrinol.* **33**, 322–328.

Hsu R. Y. and Lardy H. A. (1969) Malic enzyme. In *Methods in Enzymology* (Edited by Lowenstein J. M.), Vol. 13, pp. 230–235. Academic Press, New York.

Kafri I., Rosebrough R. W., McMurtry J. P. and Steele N. C. (1988) Corticosterone implants and supplemental dietary ascorbic acid effects on lipid metabolism in broiler chicks. *Poultry Sci.* **67**, 1356–1359.

King D. B. and Scanes C. G. (1986) Effect of mammalian growth hormone and prolactin on the growth of hypophysectomized chickens. *Comp. Biochem. Physiol.* **86A**, 29–34.

Kuhn E. R., Verheyen G., Chaisson R. B., Huts C., Huybrechts L., Van De Steen P. and Decuypere E. (1987) Growth hormone stimulates the peripheral conversion of thyroxine into triiodothyronine by increasing the liver 5′-monodeiodinase activity in the fasted and normal fed chicken. *Horm. Metab. Res.* **19**, 304–308.

Leung F. C., Yaylor J. E. and Vaniderstein A. (1986) Purified chicken growth hormone (GH) and a human pancreatic GH-releasing hormone increase body weight in chickens. *Endocrinology* **118**, 1961–1965.

Magri K. A., Gopinath R. and Etherton T. E. (1987) Inhibition of lipogenic activities by porcine growth hormone (pGH). *J. Anim. Sci.* **65** (suppl. 1), 258.

Marsh J. A., Gause W. C., Sandhu S. and Scanes C. G. (1984) Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. *Proc. Soc. Exp. Bio. Med.* **175**, 351–360.

Martin R. J. and Herbein J. H. (1976) A comparison of the enzyme levels and *in vitro* utilization of various substrates for lipogenesis in pair-fed lean and obese pigs. *Proc. Soc. Exp. Biol. Med.* **151**, 231–235.

May J. D. and Marks H. L. (1983) Thyroid activity of selected, non-selected and dwarf broiler lines. *Poultry Sci.* **62**, 1721–1724.

Oppenheimer H. H., Coulombe P.H., Schwartz H. and Gutfield N. W. (1978) Nonlinear (amplified) relationship between nuclear occupancy by triiodothyronine and the appearance rate of hepatic glycero-phosphate dehydrogenase and malic enzyme activity in the rat. *J. Clin. Invest.* **61**, 987–997.

Peel C. J., Bauman D. E., Gorewitt, R. C. and Sniffen C. J. (1981) Effect of exogenous growth hormone on lactational performance in high yielding dairy cows. *J. Nutr.* **111**, 1662–1671.

Rosebrough R. W. and Steele N. C. (1985) Energy and protein relations in the broiler. 1. Effect of protein levels and feeding regimes on growth, body composition, and *in vitro* lipogenesis of broiler chicks. *Poultry Sci.* **64**, 119–126.

Rosebrough R. W. and Steele N. C. (1986) Effect of dietary protein status on glucose utilization by the pentose cycle in pig adipose tissue. *Nutrition Research* **6**, 275–285.

Rosebrough R. W. and Steele N. C. (1987) Methods to assess glucose and lipid metabolism in avian liver explants. *Comp. Biochem. Physiol.* **88A**, 1041–1049.

Rosebrough R. W., McMurtry J. P., Mitchell A. D. and Steele N. C. (1988) Protein and energy restrictions in the broiler chicken. 6. Effect of dietary protein and energy restrictions on carbohydrate and lipid metabolism and metabolic hormone profiles. *Comp. Biochem. Physiol.* **90A**, 311–316.

Rosebrough R. W., Steele N. C. and Frobish L. T. (1981) Effect of ketogenic diets in gestation on some characteristics of carbohydrate metabolism in fetal pig brain and liver. *Growth* **45**, 42–57.

SAS Institute, Inc. (1982) *SAS User's Guide, Statistics* (Edited by Ray A. A.). SAS Institute Inc, Cary, NC.

Scanes C. G. (1987) The physiology of growth, growth hormone and other growth factors in poultry. *CRC Critical Rev. in Poult. Biol.* **1**, 51–105.

Scanes C. G., Decuypere E. and Marsh J. (1982) Plasma concentration of growth hormone, thyroxine and triiodothyronine during growth in dwarf strains of chickens. *Poultry Sci.* **61**, 1539–1548.

Scanes C. G., Duyka D. R. Lauterio T. J., Bowen S. J., Huybrechts L. M., Bacon W. L. and King D. B. (1986) Effect of chicken growth hormone, triiodothyronine and hypophysectomy in growing fowl. *Growth* **50**, 12–31.

Stewart P. A., Washburn K. W. and Marks H. L. (1984) Effect of the *dw* gene on growth, plasma hormone concentrations and hepatic enzyme activity in a random-bred population of chickens. *Growth* **48**, 59–73.

Tarlow D. M., Watkins P. A., Reed R. E., Miller R. S., Zwergel E. E. and Lane M. D. (1977) Lipogenesis and the synthesis of very low density lipoprotein by liver cells in nonproliferating monolayer culture. *Cell Biol.* **73**, 332.

Vasilatos-Younken R. (1986) Preparation and culture of dispersed avian pituitary cells, and age-related changes in pituitary weight and growth hormone content. *Gen. Comp. Endocrinol.* **64**, 99–106.

Vasilatos-Younken R. and Zarkower P. G. (1987) Age-related changes in plasma immunoreactive hormone secretory patterns in broiler pullets. *Growth* **51**, 171–180.

Vasilatos-Younken R., Cravener T. L., Cogburn L. A., Mast M. G. AND Wellenreiter R. H. (1988) Effect of pattern of administration on the response to exogenous, pituitary-derived chicken growth hormone by broiler-strain pullets. *Gen. Comp. Endocrinol.* **71**, 268–283.

Walton P. E., Etherton T. D. and Evock C. M. (1986) Antagonism of insulin action in cultured pig adipose tissue by pituitary and recombinant porcine growth hormone, potentiation by hydrocortisone. *Endocrinology* **118**, 2577–2581.

Walton P. E. and Etherton T. D. (1986) Stimulation of lipogenesis by isulin in swine adipose tissue. Antagonism by porcine growth hormone. *J. Anim. Sci.* **62**, 1584–1595.